

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Stephen RICHMOND et al.
Title: ANIMAL CELL COLONY
PICKING APPARATUS AND
METHOD
Appl. No.: 10/631,845
Filing Date: 8/1/2003
Examiner: Nathan Andrew Bowers
Art Unit: 1797
Confirmation 1041
Number:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Stephen Richmond, hereby declare and state as follows:

1. I am a British citizen and have worked for Genetix Ltd. since 1999 as a research scientist and software developer. My scientific experience covers a wide range of techniques, including molecular biology, yeast two-hybrid screening and cell biology. I was involved on the project teams developing the ClonePix, CloneSelectImager and CellReporter; all cell-based instruments. A copy of my Curriculum Vitae is attached as Exhibit A.
2. I provide the following statements, which I understand may be used to support the above-captioned application. The opinions expressed here are based on my knowledge and experience in the field.
3. I am thoroughly familiar with the above-captioned application and pending claims as I am a named inventor.

4. The pending claims relate to a method and apparatus for the automated picking of animal cell colonies.

A. Benefits of the claimed invention

5. One feature of the claimed cell picking method and apparatus which was not provided by cell picking methods and apparatus available prior to 1 August 2003 is the ability to pick up an entire cell or cell colony from a source plate (or other biological container) without disrupting the cell or cell colony. This feature provides several significant advantages:

- i. it is much more likely that viable cells from every clone present in the source plate will survive picking and thereby be represented in the destination plate to which they are transferred;
- ii. in the case of picking single cells, it is much less likely that a cell will be killed by the picking operation described herein, thus maintaining the complexity of the population;
- iii. attributes of the cell or cell colony will be maintained in the destination plate after picking, so that attributes already measured in the source plate before picking are maintained after picking, such as fluorescence level (which may represent productivity) and colony size (which may represent a specific cell type). This is important, since the cell or cell colony will in many cases have been selected for picking based on it having high performance in respect of one or more attributes measured in the source plate.
- iv. no cells from a picked colony will remain in the source plate, which if left behind would represent a potential contamination source for the remaining cell colonies;
- v. no cells will be deposited on the exterior of the pin in the course of picking, so that contamination of another cell colony in a subsequent picking operation is much less likely.

6. This feature is achieved by employing a picking head which includes a hollow pin having an inside diameter that is larger than a colony to be picked. This pin configuration is described in independent method claim 1 as follows: “a) providing a picking head comprising at least one hollow pin having an inside diameter suitable for picking animal cell colonies of a size that is smaller than said inside diameter.” (Emphasis added).

7. This pin configuration is described in independent apparatus claim 10 as follows:
“wherein the picking head comprises at least one hollow pin, the at least one hollow pin comprising an inner pin and an outer pin, wherein the inner pin is recessed axially inside an end of the outer pin, wherein the inner pin has an inside diameter, and wherein the animal cell colonies being picked have a size that is smaller than inside diameter of the inner pin.”
(Emphasis added).

B. The claimed invention is not taught or suggested by the cited prior art

9. I have read the Office Actions mailed February 3, 2009 and November 12, 2009, in which the Patent Examiner states that claimed method and apparatus are obvious in light of the disclosures of two primary references: U.S. Patent Publication No. 2003/0179916 to Magnuson ("Magnuson") and U.S. Patent No. 6,146,881 to Hering ("Hering"). I have read both of the primary references.

10. I have also read the three secondary references cited in the February 3, 2009 Office Action and the November 12, 2009 Office Action which allegedly render obvious certain elements of the dependent claims. The cited references are U.S. Patent Publication No. 2001/0019845 to Beinert, U.S. Patent No. 4,210,724 to Sogi, and U.S. Patent No. 6,064,754 to Parekh.

11. A person skilled in the art would not conceive of the claimed method and apparatus by reading Magnuson and Hering, or by reading Magnuson and Hering in conjunction with Beinert, Sogi or Parekh.

12. Magnuson teaches a cell picking apparatus based on hollow pin aspiration. However, Magnuson is emphatic in teaching that the diameter of the hollow pin must be smaller than the colony or cell to be picked. This requirement is necessary to enable the pin in Magnuson to form an "essentially airtight seal" with the colony or cell. Magnuson at paragraph [0127].

13. Experimental example 2 of Magnuson illustrates and underscores the requirement of a picking tip diameter which is smaller than the colony. Example 2 tests two different picking tip diameters: 0.2 mm and 0.4 mm. The 0.2 mm tip was smaller than the colony to be picked, while the 0.4 mm diameter tip was not smaller than the colony to be picked. See Magnuson at paragraph [0172]. In the experiment, the 0.2 mm tip easily picked a portion of the colony with very little vacuum, while the 0.4 mm picking tip failed to pick up the colony at all. According to Experimental Example 2, because "a good seal could not be maintained between the edge of the

pipette and the colony...a large amount of medium was aspirated instead of the colony.”
Magnuson at paragraph [0172].

14. Magnuson demonstrates and emphasizes that to be functional, the Magnuson hollow pin aspiration methods and device require that the picking tip diameter be smaller than the colony or cell to be picked. Accordingly, a skilled and creative artisan, after having read Magnuson, would not contemplate a hollow pin aspiration scheme in which the picking tip diameter was larger than the colony or cell to be picked. Magnuson makes clear that such a configuration does not work.

15. Hering describes apparatuses and methods for treating or handling cells in a liquid bath. Hering describes the use of a “perfusion ring” in sealed contact with the floor of the bath to isolate a portion of the liquid. *See* Hering at abstract; col. 2, lines 43-45; col. 4, lines 48-51. A conventional syringe or pipette (“liquid conveying device”) is used to withdraw fluid or cells from the contents inside the perfusion ring, or to add fluid to the contents inside the perfusion ring. *See* Hering at col. 2, lines 57-58; col. 3, lines 44-51. The syringe or pipette tip can be poked through the perfusion ring wall if the perfusion ring is made of a soft, elastic material. *See* Hering at col. 8, lines 7-13. The syringe or pipette tip can also be inserted into a “holding member” formed on the perfusion ring. *See e.g.*, Hering at col. 2, line 57. The function of the perfusion ring is to isolate the liquid inside the perfusion ring from the rest of the bath during cell handling or treatment. *See e.g.*, Hering at col. 1, lines 5-9.

16. Unlike Magnuson, Hering does not describe an automated colony picking apparatus.

17. The only element of the Hering device that could reasonably be used to aspirate cells or cell colonies is the liquid conveyance device (*e.g.*, pipette tips, syringe needles).

18. Assuming *arguendo*, that at least some of the liquid conveying devices described in Hering have a diameter larger than that of a cell colony (*e.g.*, pipette tips), the skilled and creative artisan would not see fit to incorporate a device with such a diameter into the automated colony picking apparatus of Magnuson. Simply, Magnuson clearly teaches that such a modification will not work.

19. Hering describes a perfusion ring having a diameter larger than a colony to be picked (*see e.g.*, Hering at col. 7, lines 58-63). However, the perfusion ring of Hering does not itself aspirate cells or cell colonies.

20. Incorporation of the perfusion ring of Hering to the device of Magnuson would not provide the skilled and creative artisan with the presently claimed method or apparatus: the diameter of the picking pin of Magnuson must still be smaller than a colony to be picked.

21. In addition, a perfusion ring would serve no useful purpose when incorporated into the Magnuson device. As previously noted, the picking pin of Magnuson forms “an essentially airtight seal” with the colony or cell to be picked. Magnuson at paragraph [0127]. When the required “essentially airtight seal” is formed between the picking pin and the colony, there would be no need to isolate medium inside of a perfusion ring because an isolating seal is inherent in the successful Magnuson picking process.

22. If the picking pin of Magnuson does not form such a seal on the colony, medium is aspirated instead of the colony, as described in Example 2 of Magnuson. A perfusion ring would provide no benefit with respect to aiding or ensuring colony aspiration. The medium surrounding the colony would still be aspirated with or without a perfusion ring.

23. Also, the addition of a perfusion ring to the picking pin of Magnuson (adding an outer ring to the Magnuson picking pin which forms a seal with the bottom of the plate or sample well) would likely disrupt or smear the colony as the picking head was moved to pick up the various portions of an entire colony. To pick an entire colony, the picking pin of Magnuson is “moved incrementally to aspirate the entire colony.” Magnuson at paragraph [0170]. Thus, both the picking pin and the perfusion ring would have to move incrementally to pick up an entire colony. The incremental movement of the perfusion ring across the colony and up and down in the medium with the picking pin would not only disrupt the medium, but would also likely smear or disrupt the chosen colony. Even if the perfusion ring was larger than the selected colony, the medium in which the colony was growing – be it solid, semi-solid or liquid – would be

disrupted, thereby increasing the likelihood of disrupting the colony and decreasing the likelihood of forming the required "essentially airtight seal" between the picking pin and the chosen colony.

24. Because the method and apparatus of the claimed invention do not require the picking pin to form an "essentially airtight seal" with the colony to be picked, and do not require the picking pin to be moved incrementally to pick an entire cell colony, a picking head as described in claim 10 of the present application (*e.g.*, including an inner and an outer pin) would not encounter the problems described above.

25. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that willful, false statements may jeopardize the validity of the application or any patent issued thereon.

22nd Feb, 2010
DATE

S.A. Richmond
Stephen Richmond

EXHIBIT A

Stephen Richmond

Genetix Ltd

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Current Position

Software developer with Genetix Ltd; a company providing systems imaging systems for clinical diagnosis and a range of imaging, bacterial picking and cell colony picking systems for research.

Achievements

- Currently working as a software developer on the team developing the Mk3 QPix series. A range of automated bacterial colony pickers.
- Software developer on the team that developed the CellReporter. A high-throughput system for imaging and analysing high-throughput cell-based assays.
- Biologist on the team that developed the CloneSelectImager. A system for high-throughput white-light imaging of cell colonies growing in microplates. The image analysis software monitors cell growth and determines monoclonality.
- Project leader and biologist for the team that developed the ClonePix. An instrument that automatically images and detects cell colonies growing in petri dishes or microplates, then picks them into microplates by aspirating each entire colony into a needle.
- Developed the prototype for the QPEXpression. The instrument spreads bacterial transformation mixes onto agar such that, following overnight incubation, individual colonies grow. The QPEXpression then automatically images, detects and picks the colonies into microplates.
- Biologist on the team that developed the MegaMate. An automated, high-throughput system for performing yeast two-hybrid screens.

EXHIBIT A

Experience

- 2007 - Software developer with Genetix Ltd.
- 1999 – 2007 Research scientist with Genetix Ltd.
- 1992 – 1999 Full time education at Lancaster University (see Education section).
- 1977 – 1992 Dairy herd manager (Beechwood Farms Ltd, Cockerham, Lancaster, Lancs). Established and managed a high performance, 160-cow dairy herd in addition to other responsibilities on the farm.
- 1976 – 1977 Farm worker for Mr. A. Barker, Warton Hall Farm, Warton, Carnforth, Lancs.

Education

- 2005 Open University courses: M150 Data, Computing and Information, M255 Object-Oriented Programming with Java, MST121 Using Mathematics, TT281 The Client-side of Application Development, TT282 The Server-side of Application.
- 2001 – 2003 MSc Bioinformatics modules (Manchester University on-line course); C for Bioinformatics, Java for Bioinformatics, BioComputing.
- 1995 – 1999 PhD Biological Sciences, Lancaster University. Isolation of fatty acid desaturase genes from *Picea abies* and investigation of their role in winter-hardening.
- 1995 BSc(Hons) Biological Sciences, Lancaster University (1st class)
- 1992 *Open College B unit*: Biology (83%) (night class).
- 1989 A-level Geography (D) (night class).
- 1976 *A-levels*: Biology (B), Physics-with-Maths (B), Chemistry (D), Geography (D), General Studies (B).

EXHIBIT A

1974 *O-levels*: Maths, Chemistry, Physics, Latin, French, Geography, English Language, English Literature.

Personal details

Date of birth: 16th June 1958.

Poster Presentations

SBS Meeting, 2006. CloneSelect Imager: automated imaging of cells in microplates. Steve Richmond, Laura Moody, Chris Waterhouse, Ian Taylor, Julian F. Burke.

Molecular Medicine Tri-Conference, 2006. Identification and picking of differentiated and undifferentiated mouse embryonic stem cell colonies: an automated system. Steve Richmond, Chris Mann, Ian Taylor, Julian F. Burke.

Drug Discovery Technology and Development meeting, 2005. A New Technology for Cell Line Maintenance in Microplates. Steve Richmond, Chris Mann, Susie Bercowicz, Aaron Figg, Sky Jiang, Ian Taylor, Julian F. Burke.

European Society for Animal Cell Technology meeting, 2005. CloneSelect: an automated cell management system. Steve Richmond, Chris Mann, Susie Berkowitz, Ian Taylor, Julian F. Burke.

SBS Meeting, 2005. CloneSelectImager: an automated imaging system for cells in microplates. Steve Richmond, Susie Berkowicz, Chris Mann, Aaron Figg, Sky Jiang, Ian Taylor, Julian F. Burke.

HUPO meeting, 2004. Automation of the protein expression workflow: high-throughput spreading of transformation mixes. Steve Richmond, Julian F. Burke, Laura Moody, Chris Mann, Ian Taylor.

EXHIBIT A

LabAutomation meeting, 2004. PCR Reaction Dispensing with the aliQuot. Felicity Rowley, Annabel Jenner, Steve Richmond, Mark Truesdale, Sky Jiang, Sarah Stephens, Julian Burke.

Gateway Users Meeting, 2004. Automated bacterial streaking and data tracking for Gateway transformation reactions on Genetix robots. Steve Richmond, Laura Moody, Ian Taylor, Julian F. Burke.

43rd Annual Meeting, The American Society For Cell Biology, 2003. The ClonePix: an automated solution for high-throughput picking of mammalian cell colonies. S. Richmond, C. Mann, I. Taylor, J. F. Burke.

BioChem. Society meeting, 2003. High Throughput Approaches to Microarray and Proteomic Analysis. Julian F. Burke, Chris Mann, Steve Richmond, Sarah Stephens, Mark Truesdale.

International Council of Electrophoresis Societies, 2003. Verification of automated yeast 2-hybrid screening on the MegaMate system. Steve Richmond, Chris Sanderson, Ben Lehner, Chris Mann, Julian F. Burke.

PAG meeting, 2003. Application of automated colony pickers to rapid sequencing template preparation from DNA libraries. Alysia Hallam, Therese Nestor, Steve Richmond, Sarah Stephens and Julian F Burke.

HUPO meeting, 2002. Scaling up Automated Protein Excision from 1D and 2D gels. Chris Mann, Andrew Board, James Colehan, Robert Woodrough, Steve Richmond and Sarah Stephens.

EXHIBIT A

HUPO meeting, 2002. High-throughput mapping of protein-protein interactions:
automation of the yeast two-hybrid system

Steve Richmond, Kelly Gregan, Kevin Russell, Chris Mann, Julian F. Burke.

LabAutomation meeting, 2002. Automation of the yeast two hybrid system. Steve
Richmond, Kelly Gregan, Julian Burke.

Human Genome Meeting, 2002. High-throughput yeast two hybrid screening
Steve Richmond, Kelly Gregan, Julian Burke.

Human Genome Meeting, 2002. Ultra-Fast 2D-Gel Protein Excision: The gelPix System.
Chris Mann, Andrew Board, James Colehan, Steve Richmond, Sarah Stephens.

HUGO meeting, 2001. Storage of gene libraries as glycerol stocks in 1536-well plates
Steve Richmond, Andrew Trowbridge and Mark Reid.